Biochimica et Biophysica Acta 1833 (2013) 1665–1671

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Three calcium-sensitive genes, *fus*, *brd3* and *wdr5*, are highly expressed in neural and renal territories during amphibian development ☆

A. Bibonne [a,b,1], I. Néant [a,b,1,2], J. Batut [a,b], C. Leclerc [a,b,2], M. Moreau [a,b,2], T. Gilbert [a,c,2,*]

[a] Université Toulouse 3, Centre de Biologie du Développement, Toulouse, F-31000, France
[b] CNRS, UMR 5547, Toulouse, F-31000, France
[c] INSERM, ADR Midi Pyrénées, Toulouse, F-31000, France

ARTICLE INFO

Article history:
Received 6 November 2012
Received in revised form 12 December 2012
Accepted 14 December 2012
Available online 31 December 2012

Keywords:
Calcium-dependent target gene
Development
Neurogenesis
Pronephros
Kidney
Xenopus

ABSTRACT

Numerous Ca^{2+} signaling events have been associated with early development of vertebrate embryo, from fertilization to organogenesis. In *Xenopus laevis*, Ca^{2+} signals are key regulators in the earliest steps of the nervous system development. If neural determination is one of the best-characterized examples of the role of Ca^{2+} during embryogenesis, increasing literature supports a determining role of organogenesis and differentiation. In blastula the cells of the presumptive ectoderm (animal caps) are pluripotent and can be induced toward neural fate with an intracellular increase of free Ca^{2+} triggered by caffeine. To identify genes that are transcribed early upon Ca^{2+} stimuli and involved in neural determination, we have constructed a subtractive cDNA library between neuralized and non-neuralized animal caps. Here we present the expression pattern of three new Ca^{2+}-sensitive genes: *fus* (*fus*ed in sarcoma), *brd3* (bromodomain containing 3) and *wdr5* (WD repeat domain 5) as they all represent potential regulators of the transcriptional machinery. Using in situ hybridization we illustrated the spatial expression pattern of *fus*, *brd3* and *wdr5* during early developmental stages of *Xenopus* embryos. Strikingly, their domains of expression are not restricted to neural territories. They all share a specific expression throughout renal organogenesis which has been found to rely also on Ca^{2+} signaling. This therefore highlights the key function of Ca^{2+} target genes in specific territories during early development. We propose that Ca^{2+} signaling through modulation of *fus*, *brd3* and *wdr5* expressions can control the transcription machinery to achieve proper embryogenesis. This article is part of a Special Issue entitled: 12th European Symposium on Calcium.

1. Introduction

During embryonic development, a rise in intracellular Ca^{2+} is becoming a widespread trigger to direct cell stemness towards a specific tissue fate. Numerous developmental Ca^{2+} signaling events have been associated with the early development of vertebrate embryos, from meiosis, through fertilization, differentiation and organogenesis [1–6]. In *Xenopus laevis*, Ca^{2+} signals are important regulators in the earliest steps of nervous system development which relies on neural induction [1,7]. Neural induction is the process by which naive ectoderm is induced towards neural pathways. In amphibian, Ca^{2+} is a necessary and sufficient signal to convert the ectoderm into neuro-ectoderm [8]. Indeed, naive ectoderm isolated at blastula stage (animal cap) can be induced to differentiate into neurons and glial cells following short term culture in presence of caffeine, which triggers a rise of intracellular Ca^{2+} [9]. And this ex vivo assay was further used to characterize the Ca^{2+} signaling pathways that lead to the expression of neural specific genes.

Using this animal cap assay we have previously generated a subtractive library to identify genes specifically involved in neural induction. We have first isolated *mlp* as an early Ca^{2+}-responsive gene since it was activated within 30 min upon Ca^{2+} transient signals [10]. It is expressed in neural territories from gastrula to tailbud stage. Then another Ca^{2+} responsive gene, *prmt1b*, has been discovered using this strategy. It codes for the Protein aRginine MethylTransferase 1b that turned out to be a key modulator of neural fate determination in the amphibian embryo [11,12]. More recently we have reported that *p54nrb* is an additional Ca^{2+}-responding gene involved in anterior neural patterning [12]. We found that a specific down regulation of this RNA-binding protein leads to neurogenesis defects and ocular abnormalities [12]. The major expression pattern of these three Ca^{2+}-responding genes is found in the developing nervous system, and it is conserved in mammalian

☆ This article is part of a Special Issue entitled: 12th European Symposium on Calcium.

* Corresponding author at: Centre de Biologie du Développement, CNRS UMR 5547, Université Toulouse 3 — Paul Sabatier, Bat 4R3, 118 Route de Narbonne, 31062 Toulouse Cedex 9, France. Tel.: +33 561 558 695; fax: +33 561 556 507.
E-mail address: thierry.gilbert@univ-tlse3.fr (T. Gilbert).

[1] Both authors contributed equally.

[2] GDRE 731 "Ca^{2+} toolkit coded proteins as drug targets in animal and plant cells".

0167-4889/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbamcr.2012.12.015

embryos [13–15]. If neural determination is one of the best characterized examples of the role of Ca^{2+} during embryogenesis, emerging literature reports its previously unrecognized role in organogenesis and differentiation such as muscle formation [16] and kidney development [17,18].

How Ca^{2+} signaling can achieve these pleiotropic effects during embryogenesis is still poorly understood. In this study we reveal the expression pattern of three additional Ca^{2+} early target genes isolated from our original screen, *fus* (*fus*ed in sarcoma), *brd3* (bromodomain containing 3) and *wdr5* (WD repeat domain 5) during *Xenopus laevis* development. They all represent potential regulators of the transcriptional machinery in early *Xenopus* development. Fus belongs to a family of protein known as FET, which includes Ewing's sarcoma and TATA-binding protein-associated factor15, and encodes a RNA-binding protein. Fus regulates various aspects of RNA metabolism and processing, as well as micro RNA biogenesis [19]. In human, RNA processing alterations caused by *FUS* mutations can trigger premature degeneration of motor neurons in amyotrophic lateral sclerosis [20]. Interestingly the nuclear-cytoplasmic shuttling of FUS can be modulated by PRMT1 [21]. During early *Xenopus* development, *fus* is necessary for proper mRNA splicing of key developmental regulatory genes [22]. The second calcium target gene identified in this study, *brd3*, encodes a member of the BET (Bromodomain and Extra Terminal motif) proteins family. Bromodomain-containing proteins are acknowledged as key epigenetic regulators of gene transcription. These proteins can selectively interact with acetylated lysines on histones to allow transcription [23], but they can also interact with acetylated transcription factors to promote their stable association with specific DNA sequences [24]. Finally, *wdr5* is a core member of the trithorax complex, acting as a "presenter" of the lysine 4 residue of histone H3 for its specific methylation [25,26]. Recently, it has been shown that Wdr5 can specifically interact with Oct4 to serve as a key mediator of self-renewal and reprogramming via the embryonic stem cell core transcriptional network [27].

Here we analyzed the expression patterns of these three Ca^{2+} early target genes in *Xenopus* development. On the contrary to *prmt1b* and *p54nrb*, the expression domains of *fus*, *brd3* and *wdr5* are not restricted to neural territories but are also present in other tissues, principally in the pronephros. It should be mentioned that *mlp* previously isolated from our screen is also expressed in pronephric territories [28]. By specifically controlling different aspects of the transcriptional machinery, we propose that these Ca^{2+} target genes could control the proper timing of organogenesis during embryogenesis.

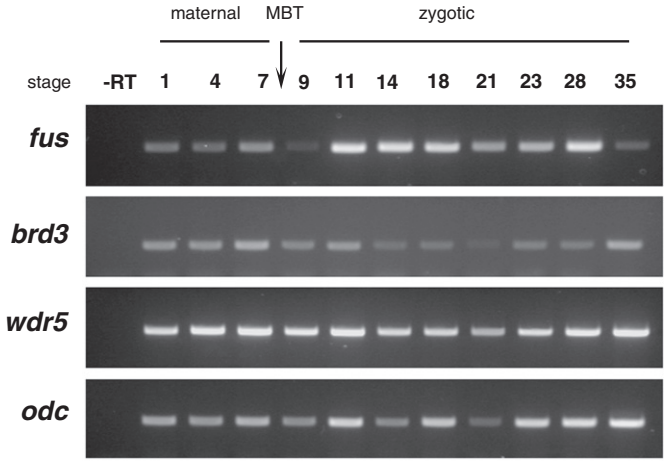

Fig. 1. Temporal expression profiles of *fus*, *brd3* and *wdr5* during early development. RT-PCR analysis of RNA extracted from embryos at the indicated stages. PCR on RNA without reverse transcription was performed to check the absence of genomic DNA (lane—RT). *odc* was used as a loading control. In *Xenopus laevis* the mid-blastula transition (MBT) occurs 6 h after fertilization, at stage 8 and marks the transition between maternal and zygotic gene expression.

2. Materials and methods

2.1. Embryos and animal caps

Xenopus laevis eggs were collected, in vitro fertilized, and embryos were cultured under standard procedures at 21 °C in amphibian medium [10]. Embryos were staged according to Nieuwkoop and Faber [29]. Animal caps, corresponding to ectodermal territory, were dissected at stage 8–8.5 and cultured in absence (control) or presence (induced) of 10 mM of caffeine (Sigma) to trigger Ca^{2+} release, for kinetics from 15 to 60 min.

2.2. Suppression subtractive cDNA library

0.6 μg of total RNA from control untreated (i.e., ectodermal) and caffeine-treated (i.e., neuralized) animal caps cultured for 15 to 60 min was extracted and subjected to the PCR-select cDNA subtraction kit, followed by PCR-select differential screening kit according to Clontech instructions, as described [10]. Briefly, we obtained four populations of cDNA: initial control cDNA population from untreated animal caps; initial caffeine-induced cDNA population; subtracted "sense" cDNA population corresponding to an enrichment of specific Ca^{2+}-induced cDNAs minus control cDNAs; and subtracted "reverse" cDNA population corresponding to cDNA from control animal caps minus caffeine-induced animal caps. The cDNA fragments specific of the Ca^{2+}-induced condition were inserted in pGEMT-Easy vector, bacterially amplified and spotted on nylon membranes in 4 replicates. To screen the inserts specific to Ca^{2+} induction, the 4 membranes were hybridized independently with ^{32}P-radiolabeled probes issued from the 4 previously described cDNA populations. Identification of the fragments of interest was performed by sequencing the corresponding cDNA insert, and compared to Genbank databases.

2.3. RT-PCR

Total RNA was isolated using Qiagen RNeasy mini columns. Random hexamer primed cDNA was synthesized using SuperScript II (Invitrogen) followed by standard PCR methods. Forward and reverse primer sets were as follows: *fus* (NM_001086914), 5′-ATGGATTCCAGGGGAGA TAGG-3′ (F) and 5′-TAAAATTCACAGGCGCACAG-3′ (R) corresponding to 1608–1789 bp region; *brd3* (NM_001097126), 5′-CTGTTTTGGCCAAGAT CACAA-3′ (F) and 5′-TACAGGGGAAAGCATGAAGG-3′ (R) corresponding to region 3306–3500 bp; and *wdr5* (NM_001093505) 5′-AGACCAAATAA GCCACCTGCCGTTGC-3′ (F) and 5′-GACACGAGGCGTAAGCAATACAATAC-3′ (R) to region 2157–2530 bp. Annealing temperature was 55 °C. *odc* (ornithine decarboxylase) used as housing keeping gene and loading control was amplified with 5′-TGGATTTCAGAGACCAAC-3′ (F) and 5′-CCAAGGCTAAAGTTGCAG-3′ (R) primers corresponding to the 1486–1717 region. The absence of genomic contamination was checked with *odc* amplification of the RNA samples without reverse transcriptase.

2.4. In situ hybridization

Probe synthesis for *fus*, *brd3* and *wdr5* was performed using PCR in order to generate about 800 nt long antisense digoxigenin-labeled riboprobes. cDNA templates for *fus*, *brd3* and *wdr5* were first generated using the following forward and reverse primers as indicated here: *fus*, 5′-GCAGCCAAGCTCTTATGGAC-3′ (F), 5′-TCGCCAGTTTCTTCGT CTTT-3′ (R); *brd3*, 5′-TCAAGAACCCGATGGATCTC-3′ (F), 5′-TTCCCG GGATTGTATGATGT-3′ (R); and *wdr5*, 5′-GTTGGGAATATCCGATGTG G-3′ (F), 5′-ATGAGGATTGTTCCCCTTCC-3′ (R). PCR was run at 63 °C in 1.5 mM MgCl2 and for 30 cycles. The following T7 promoter sequence, 5′-CGATGTTAATACGACTCACTATAGGG-3′, was added in front of the reverse primer of interest in order to generate in a second PCR run templates ready for in vitro transcription. Primers were designed using Primer 3 software [30]. In situ hybridization on whole

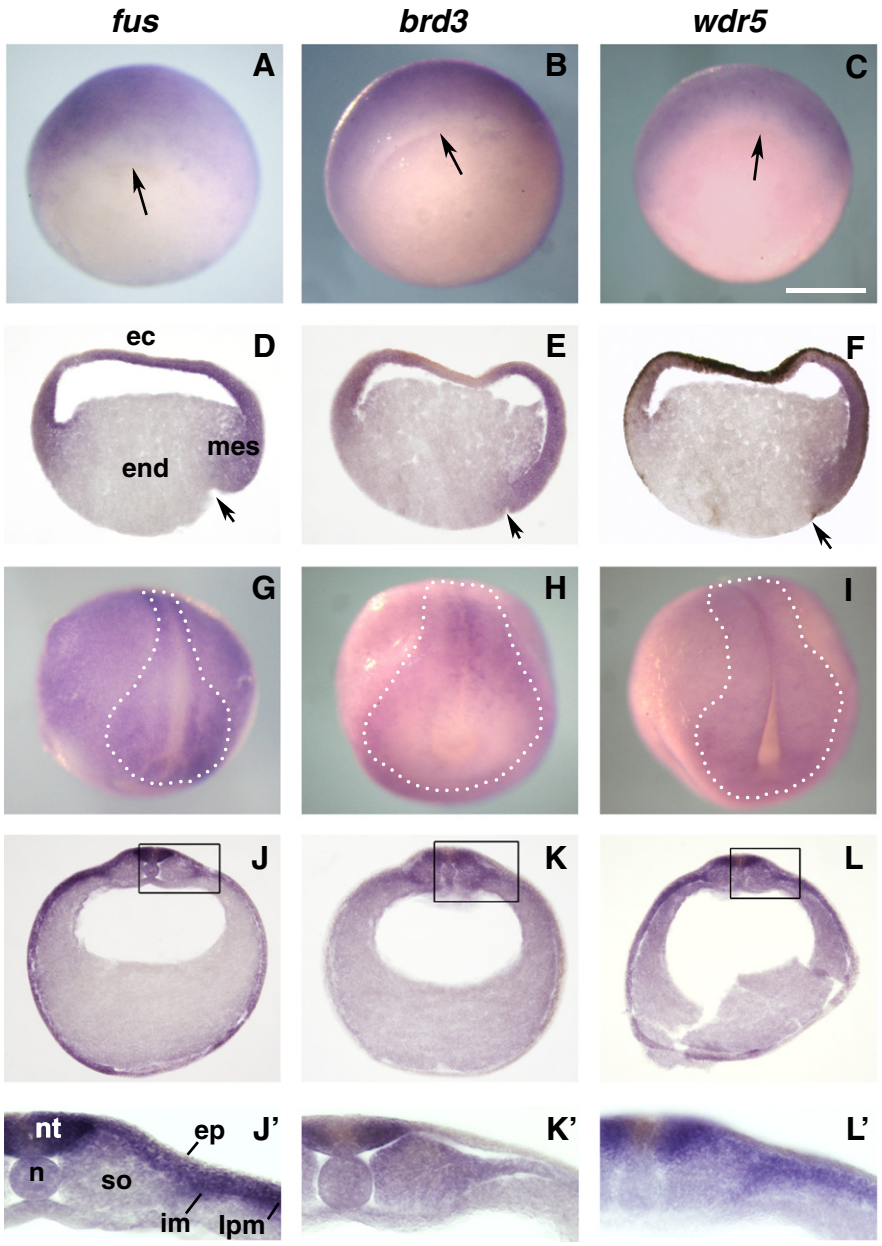

Fig. 2. Spatial expression patterns of *fus*, *brd3* and *wdr5* mRNA during *Xenopus laevis*' early development. In situ hybridization is performed on embryos and sections at gastrula and neurula stages. Photomicrographs of whole mounts and sections for *fus* (left panels), *brd3* (middle panels) and *wdr5* (right panels) are shown. (A–C) At early gastrula (dorsal view, arrows indicate the position of the blastoporal lip) *fus*, *brd3* and *wdr5* transcripts are detected in the ectoderm and the mesoderm. (D–F) This pattern is confirmed by section analysis (sagittal section dorsal is on the right, arrows point to the dorsal blastoporal groove) which shows labeled ectoderm (ec) with no difference between the dorsal and the ventral sides and in the dorsal mesoderm (mes). The endoderm (end) is not labeled. (G–H) Whole-mounts at early neurula stage (anterior view, dorsal side up), expression is restricted to neural territories as indicated by the dotted areas. (J–L) Transversal section analysis (section is a trunk level, dorsal is up) shows that *fus*, *brd3* and *wdr5* are expressed in the neural tube (nt). Enlargement of respective sections (J'–L'), the epidermal ectoderm (ep) is labeled for *fus* (J') but not for *brd3* and *wdr5* (K', L'). A strong expression is present in the intermediate mesoderm (im) and in the lateral plate mesoderm (lpm) for *fus* and *wdr5* (J', L') while *brd3* is clearly expressed in the somitic mesoderm (so). Abbreviations: ec; ectoderm, end; endoderm, ep; epidermal ectoderm, im; intermediate mesoderm, lpm; lateral plate mesoderm, mes; mesoderm, n; notochord, nt; neural tube, so; somitic mesoderm. Scale bars 0.5 mm in (A–L).

embryo and on 70 μm-thick vibratome sections was performed as previously described [11].

3. Results

3.1. Identification of fus, brd3 and wdr5 by differential screening of subtractive library

In order to identify genes that are specifically induced by a rise of intracellular Ca^{2+}, we have constructed a subtractive cDNA library using the animal caps in *Xenopus laevis*. In this model, untreated animal caps spontaneously evolved towards an epidermal fate whereas a caffeine treatment, as short as 15 min, is sufficient to induce a release of intracellular $Ca^{2+}$and to trigger neural fate of the ectoderm (for review see [1]). To isolate the early genes that precociously respond to Ca^{2+}, we first prepared animal caps from stage 8 embryos and cultivated them in absence (control) or in the presence (induced) of caffeine to trigger a burst of intracellular Ca^{2+} concentration as previously described [11]. A suppressive subtractive hybridization (SSH) was performed between these Ca^{2+}-induced and non induced animal caps to generate a cDNA library enriched in cDNA fragments corresponding to genes specifically expressed in the Ca^{2+}-induced ectoderms. Clones of the subtracted library were loaded on nylon membranes and these deposits were

Table 1
Summary of *fus*, *brd3* and *wdr5* expression patterns in the developing *Xenopus* embryos.

	fus	*brd3*	*wdr5*
Gastrula			
Ectoderm	+++	+++	+++
Mesoderm	++	(+)	+
Endoderm	−	−	−
Neurula			
Neural tube	+++	+	++
Notochord	+	(+)	−
Archenteron roof	(+)	−	(+)
Epidermal ectoderm	++	−	−
Somitogenic mesoderm	−	(+)	(+)
Intermediate mesoderm	+++	(+)	++
Lateral plate mesoderm	+++	(+)	++
Tailbud			
Cement gland	−	++	+
Telencephalon	(+)	(+)	(+)
Mesencephalon	+	+	+
Rhombencephalon	+	+	+
Spinal cord	+++	+	+
Optic cup/Eye	++	+	++
Otic vesicle	+	+	(+)
Branchial arches	++	(+)	+
Heart	+	(+)	(+)
Somite	−	−	−
Pronephros	++	++	+
Proctodeum	+	+	+
Epidermis	−	−	−
Tail bud	+++	+	+

+++, ++, +, and (+): presence of detectable ISH signal from very high (+++) to weak (+) expression.
−: absence of detectable levels of expression.

screened with specific labeled probes derived from control, induced, and subtracted populations to highlight the presence of Ca^{2+} responsive genes. Data are presented on Supplementary Fig. 1. Three isolated cDNA fragments corresponding to the genes *fus* (*fused in sarcoma*, accession number NM_001086914), *brd3* (*Bromo-domain containing 3*, accession number NM_001097126) and *wdr5* (*WD repeat 5*, accession number NM_001093505) were further studied as they represented the most pertinent candidates with Ca^{2+}-dependent expression as the ones previously described [10–12].

The expression of *fus*, *brd3* and *wdr5* during early *Xenopus laevis* development was analyzed by RT-PCR (Fig. 1). Abundant *fus*, *brd3* and *wdr5* transcripts were observed prior the mid blastula transition (MBT), from fertilization (stage1) to early blastula (stage7) indicating a strong maternal expression. Then *fus*, *brd3* and *wdr5* are all expressed zygotically from stage 9 onwards.

3.2. Spatial expression patterns at gastrula and neurula stages

As Ca^{2+} signaling is a necessary pathway to instruct the competent ectoderm to the neural fate, we verified whether the three Ca^{2+}-induced genes selected here by SSH displayed an expression pattern in neural territories during early *Xenopus* development using in situ hybridization. Expression patterns of *fus*, *brd3* and *wdr5* at gastrula and neurula stages from whole mount embryo and on vibratome sections are presented in Fig. 2 and summarized in Table 1. All three transcripts were detected during gastrulation with a significant expression in animal and marginal zones (Fig. 2A–C). Sagittal sections in Fig. 2D–F reveal that *fus*, *brd3* and *wdr5* are expressed in the ectoderm with no difference between the dorsal and the ventral side. However, in the mesoderm these three genes display differential expression between the dorsal and the ventral side. In the dorsal mesoderm *fus*, *brd*3 and *wdr5* are expressed homogenously. In the ventral mesoderm *brd3* is weakly expressed and *wdr5* seems to be absent (Fig. 2E and F). No labeling was detected within the endoderm. At neurula stage, while *fus* is expressed in anterior and posterior neural territories as well as in epidermal ectoderm (Fig. 2G), the expression of *brd3* and *wdr5* transcripts is restricted to neural territories (Fig. 2H–I). It is to note that the overall level of expression of the three transcripts during gastrulation is low. In order to better analyze their specific sites of expression, in situ hybridization was then performed on transverse sections. On transverse trunk sections, *fus* staining was prominent in the neural tube as well as along the lateral plate mesoderm (Fig. 2J). Higher magnification indicates that *fus* transcripts are present in the epidermal ectoderm and in the intermediate and lateral plate mesoderm while absent in the notochord and very weakly expressed in the somitic mesoderm (Fig. 2J'). The expression patterns for *brd3* and *wdr5* reveal a strong expression in the neural tube like for *fus* (Fig. 2K, L, K' and L'). However, in contrary to *fus* no expression is detected in the epidermal ectoderm (Fig. 2K',L'). While the expression of *wdr5* is detected in the intermediate and the lateral plate mesoderm (Fig. 2L'), *brd3* is expressed at a low level in the intermediate mesoderm and seems to be absent in the lateral plate mesoderm (Fig. 2K'). In addition, *brd3* signals in the somitic region could be detected and are stronger than those derived from *fus* and *wdr5* transcripts (Fig. 2J'–L'). The spatial expression patterns at neurula stage indicate that *fus*, *brd3* and *wdr5* genes are present not only in neural territories but also in the intermediate mesoderm, a tissue from which originates the larval kidney (i.e. the pronephros).

3.3. Spatial expression patterns at tailbud and tadpole stages

Following on *Xenopus* development, we analyzed the expression of *fus*, *brd3* and *wdr5* at tailbud and tadpole stages in order to determine whether these three genes are also present in the pronephros. Data are summarized in Fig. 3 and in Table 1. At early tailbud stage, prominent expression of these three genes was found in the central nervous system (brain and spinal cord) (Fig. 3A–F). The optic cup and to a lesser extent the otic vesicle expressed also significant level of *fus*, *brd3* and *wdr5* (Fig. 3A–C). *fus* and *wdr5* transcripts are clearly present in branchial arches (Fig. 3A,C), while *brd3* is weakly expressed in this structure (Fig. 3B). The cement gland is negative for *fus* and positive for *brd3* and *wdr5*. Interestingly, examination of vibratome sections subjected to in situ hybridization for these three genes revealed that they are all expressed in the pronephros as well as in the lateral plate mesoderm (Fig. 3D–F). At late tailbud stage, a strong expression of *fus*, *brd3* and *wdr5* remained in the developing eye and otic vesicle (Fig. 3G–I). The nascent tail and proctodeum become positive for these three Ca^{2+} early target genes. However, although *fus* and *wdr5* are still highly expressed in the spinal cord, the pronephric tubules and the lateral plate mesoderm (Fig. 3J,L), *brd3* expression was nearly absent in those compartments (Fig. 3K). Examination of tadpole stage embryos showed that *fus*, *brd3* and *wdr5* transcripts are present in the tail (Fig. 3M–O). An additional *fus* expression is now observed in the migrating muscle anlagen. As shown in the transverse sections at pronephric level (Fig. 3P–R), *fus* is still highly expressed in the central nervous system and the embryonic kidney (Fig. 3P) while *brd3* and *wdr5* remain faintly expressed in the spinal cord but they are no more present in the pronephric tubules (Fig. 3Q,R). From the tailbud stage, no detectable levels of *fus*, *brd3* and *wdr5* could be visualized in the somites.

Each three genes selected from the SSH library of Ca^{2+}-dependent genes exhibits a highly dynamic expression pattern during *Xenopus* development, from competent ectoderm at gastrula to the central and sensorial nervous systems, and from mesoderm to lateral plate mesoderm and its regionalized derivatives, particularly the pronephros.

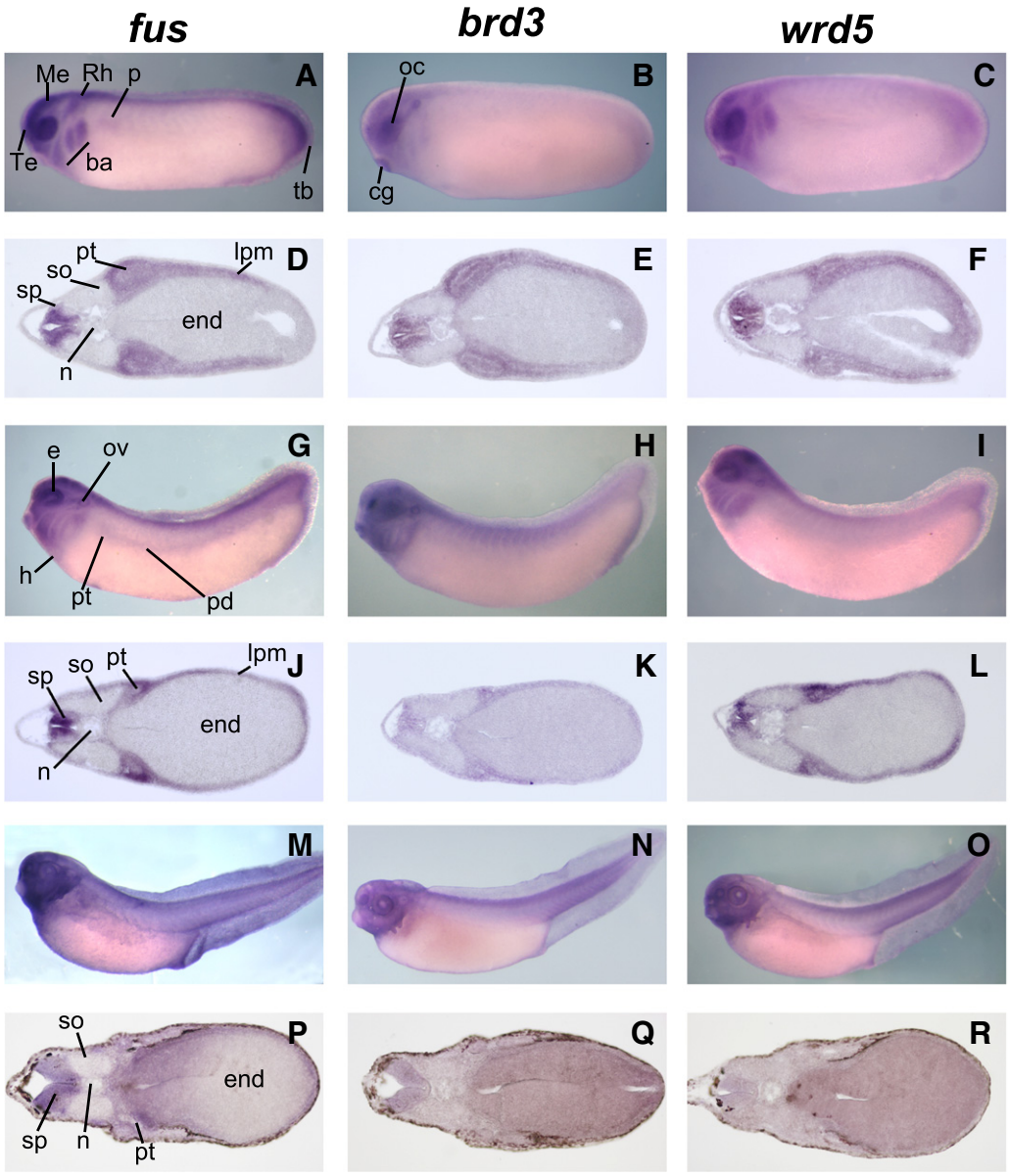

Fig. 3. Spatial expression patterns of *fus*, *brd3* and *wdr5* at taibud and tadpole stages in *Xenopus laevis*. In situ hybridization is performed on embryos and sections at early (A–F), late tailbud (G–L) and tadpole (M–R) stages. Photomicrographs of whole mounts and sections for *fus* (left panels), *brd3* (middle panels) and *wdr5* (right panels) are shown. (A–C) At early tailbud *fus*, *brd3* and *wdr5* transcripts are expressed in anterior neural structures; telencephalon (Te), mesencephalon (Me) and rhombencephalon (Rh) and in the optic cup (oc). A strong expression is detected in the branchial arches (ba) for *fus* and *wdr5* (A, C). The cement gland (cg) expressed *brd3* and *wdr5* (B, C). The tail bud (tb) and the pronephros (p) express *fus* at this stage (A). (D–E) Transversal sections at the level of the pronephros show the expression of all three transcripts in the spinal cord (sp), the pronephric tubule (pt) and the lateral plate mesoderm (lpm), and the endodermal yolk mass (end), notochord (n) and somites (so) are not labeled. (G–I) Late tailbud stage shows similar labeling patterns to the early tailbud. In addition, the pronephric tubule (pt) and duct (pd) are clearly labeled for *fus*; and the heart territory (h) for *fus* and *wdr5* (G, I). (J–L) Transversal sections at the level of the pronephros confirm these patterns of expression and reveal that the level of *brd3* transcripts decreases at this stage. (M–O) Whole-mounts and (P–R) transversal sections at pronephros level tadpole stage. Section analysis at this stage reveals that *fus* transcripts remain expressed in spinal cord (sp) and pronephric tubules (pt) *brd3* and *wdr5* are undetectable in these structures. Abbreviations: ba; branchial arches, cg; cement gland, e; eye, end; endodermal yolk mass, h; heart territory, lpm; lateral plate mesoderm, Me; mesencephalon, n; notochord, oc; optic cup, ov; otic vesicle, p; pronephros, pd; pronephric duct, pt; pronephric tubule, Rh; rhombencephalon, so; somite, sp; spinal cord, tb; tail bud, Te; telencephalon.

4. Discussion

Our subtractive cDNA library specifically enriched in Ca^{2+}-responsive early genes leads to the identification of three genes known to be involved in the modulation of the transcriptional machinery, namely *fus*, *brd3* and *wdr5*. Here we show that their expressions are spatially and temporally controlled during *Xenopus* embryogenesis. At gastrula, all three are expressed in the ectoderm and mesoderm whereas their transcripts were below the threshold of detection in the endoderm. Later on, all three transcripts are found in neural and sensorial territories (encephalon, neural tube, eye and branchial arches) and among the mesodermal derivatives, the pronephros turn out to express significant levels of each of them as soon as the renal primordium is formed. Then the expressions of *fus*, *brd3* and *wdr5* cease sequentially from early tailbud stage, with *brd3* being extinct first followed by *wdr5* at tadpole stage, whereas renal *fus* expression remains. Few data are available on their expression in other animal models. For example, the murine homolog of *fus* previously named *pigpen* is developmentally regulated during craniofacial morphogenesis with a prominent expression to proliferating regions of the lens and neural retina [31]. The expression of *brd3* is spatially restricted in mouse neonatal neocortex [32]. For *wdr5*, the drosophila homolog *wds* (*will dye slowly*) is abundantly expressed in the larval brain [33]. Besides these expected neural

expressions, *fus*, *brd3* and *wdr5* transcripts are also present during mammalian kidney development [31,34]. They are all expressed in the epithelial ureteric bud and/or the mesenchymal pool of nephron progenitors. However, if the expressions of *brd3* and *wdr5* are sustained throughout renal organogenesis, *fus* expression was not detected during the final boost of nephron formation [34].

The duality of neural and renal expressions is not uncommon during development. In fact, identical signaling pathways, including BMP, Wnt, FGF and GDNF are implicated during *Xenopus* neural and kidney development [35,36]. The requirement for Ca^{2+} signaling has also been demonstrated during the early phases of neural and pronephros development in the amphibian [1,18]. A screen aimed at uncover genes involved in acquisition of a neural identity by ectodermal cells after BMP inhibition, identified several clones which displayed expression in both nervous system and pronephros in *Xenopus laevis*. One of them, RNAbpEWS, is a member of the FET family of DNA/RNA-binding proteins, which comprises *fus* [37].

To date few experiments have tackled the function of these genes during early development. Very recently, *fus* involvement during early *Xenopus* development has been demonstrated [22]. Failure to gastrulate and defects of mesodermal differentiation upon *fus* knockdown were notably related to intron retention of FGF8, a growth factor required for early mesoderm induction [22]. Interestingly, FGF8 is also known to play a crucial role for optimal renal territory formation in mice [38]. Knockdown of *wdr5* during *Xenopus* development leads to mesodermal and endodermal patterning defects illustrated by shortened and twisted tadpoles and gut abnormalities [26] as well as to a significant decrease of the smooth muscle cell lineage [39]. The former defects were secondary to abnormal spatial regulation of *Hox* gene expression, related to a default of the methylation state by the H3K4 methyltransferase complex [26]. This further agrees with *wdr5* being a component of the mammalian SET1A/SET1B histone H3-K4 methyltransferase complexes [40]. In mice, optimal *wdr5* levels are required for induction of the osteoblast [41].

In conclusion, although chromatin remodeling and transcriptional activation during cell lineage determination is required for optimal embryogenesis, very few data are available on both spatial and temporal expression patterns of chromatin remodelers during embryogenesis. Here we provide evidence that *fus*, *brd3* and *wdr5* are present during key steps of *Xenopus* development and are spatially and temporally restricted. They are sequentially expressed during pronephros formation. If *fus* is known to be a central factor in early amphibian development, additional investigations are needed to further characterize its role during kidney formation. The same holds true for *wdr5* as a potent transcriptional activator, and *brd3* as a transcription initiation factor. Finally the occurrence of epigenetic modifications that have been recently reported during kidney development [42,43] illustrates that they are likely to be essential to establish a kidney-specific fate.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamcr.2012.12.015.

Acknowledgements

This work was supported by CNRS (Centre National de la Recherche Scientifique), GDRE731 (Groupement De Recherche Européen, CNRS) and Inserm (Institut national de la santé et de la recherche médicale). We thank Stéphane Relexans for taking care of the *Xenopus* facility.

References

[1] C. Leclerc, I. Néant, M. Moreau, The calcium: an early signal that initiates the formation of the nervous system during embryogenesis, Front. Mol. Neurosci. 5 (2012) 3.
[2] M. Moreau, J.P. Vilain, P. Guerrier, Free calcium changes associated with hormone action in amphibian oocytes, Dev. Biol. 78 (1980) 201–214.
[3] M. Moreau, C. Leclerc, P. Guerrier, Meiosis reinitiation in *Ruditapes philippinarum* (Mollusca): involvement of L-calcium channels in the release of metaphase I block, Zygote 4 (1996) 151–157.
[4] K. Swann, Y. Yu, The dynamics of calcium oscillations that activate mammalian eggs, Int. J. Dev. Biol. 52 (2008) 585–594.
[5] S.E. Webb, R.A. Fluck, A.L. Miller, Calcium signaling during the early development of medaka and zebrafish, Biochimie 93 (2011) 2112–2125.
[6] S.S. Rosenberg, N.C. Spitzer, Calcium signaling in neuronal development, Cold Spring Harb. Perspect. Biol. 3 (2011) a004259.
[7] C. Leclerc, I. Néant, S.E. Webb, A.L. Miller, M. Moreau, Calcium transients and calcium signalling during early neurogenesis in the amphibian embryo *Xenopus laevis*, Biochim. Biophys. Acta 1763 (2006) 1184–1191.
[8] C. Leclerc, S.E. Webb, C. Daguzan, M. Moreau, A.L. Miller, Imaging patterns of calcium transients during neural induction in *Xenopus laevis* embryos, J. Cell Sci. 113 (Pt 19) (2000) 3519–3529.
[9] M. Moreau, C. Leclerc, L. Gualandris-Parisot, A.M. Duprat, Increased internal Ca2+ mediates neural induction in the amphibian embryo, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 12639–12643.
[10] J. Batut, I. Néant, C. Leclerc, M. Moreau, xMLP is an early response calcium target gene in neural determination in *Xenopus laevis*, J. Soc. Biol. 197 (2003) 283–289.
[11] J. Batut, L. Vandel, C. Leclerc, C. Daguzan, M. Moreau, I. Néant, The Ca^{2+}-induced methyltransferase xPRMT1b controls neural fate in amphibian embryo, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 15128–15133.
[12] I. Neant, N. Deisig, P. Scerbo, C. Leclerc, M. Moreau, The RNA-binding protein Xp54nrb isolated from a Ca^{2+}-dependent screen is expressed in neural structures during *Xenopus laevis* development, Int. J. Dev. Biol. 55 (2011) 923–931.
[13] T. Umekage, K. Kato, A mouse brain cDNA encodes a novel protein with the protein kinase C phosphorylation site domain common to MARCKS, FEBS Lett. 286 (1991) 147–151.
[14] M.R. Pawlak, C.A. Scherer, J. Chen, M.J. Roshon, H.E. Ruley, Arginine N-methyltransferase 1 is required for early postimplantation mouse development, but cells deficient in the enzyme are viable, Mol. Cell. Biol. 20 (2000) 4859–4869.
[15] A.E. McKee, E. Minet, C. Stern, S. Riahi, C.D. Stiles, P.A. Silver, A genome-wide in situ hybridization map of RNA-binding proteins reveals anatomically restricted expression in the developing mouse brain, BMC Dev. Biol. 5 (2005) 14.
[16] S.E. Webb, A.L. Miller, Visualization of Ca^{2}+ signaling during embryonic skeletal muscle formation in vertebrates, Cold Spring Harb. Perspect. Biol. 3 (2011).
[17] T. Gilbert, C. Leclerc, M. Moreau, Control of kidney development by calcium ions, Biochimie 93 (2011) 2126–2131.
[18] C. Leclerc, S.E. Webb, A.L. Miller, M. Moreau, An increase in intracellular Ca2+ is involved in pronephric tubule differentiation in the amphibian *Xenopus laevis*, Dev. Biol. 321 (2008) 357–367.
[19] M. van Blitterswijk, J.E. Landers, RNA processing pathways in amyotrophic lateral sclerosis, Neurogenetics 11 (2010) 275–290.
[20] C. Vance, B. Rogelj, T. Hortobágyi, K.J. De Vos, A.L. Nishimura, J. Sreedharan, X. Hu, B. Smith, D. Ruddy, P. Wright, J. Ganesalingam, K.L. Williams, V. Tripathi, S. Al-Saraj, A. Al-Chalabi, P.N. Leigh, I.P. Blair, G. Nicholson, J. de Belleroche, J.M. Gallo, C.C. Miller, C.E. Shaw, Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6, Science 323 (2009) 1208–1211.
[21] M.L. Tradewell, Z. Yu, M. Tibshirani, M.C. Boulanger, H.D. Durham, S. Richard, Arginine methylation by PRMT1 regulates nuclear-cytoplasmic localization and toxicity of FUS/TLS harbouring ALS-linked mutations, Hum. Mol. Genet. 21 (2012) 136–149.
[22] D.S. Dichmann, R.M. Harland, fus/TLS orchestrates splicing of developmental regulators during gastrulation, Genes Dev. 26 (2012) 1351–1363.
[23] G. LeRoy, B. Rickards, S.J. Flint, The double bromodomain proteins Brd2 and Brd3 couple histone acetylation to transcription, Mol. Cell 30 (2008) 51–60.
[24] J.M. Lamonica, W. Deng, S. Kadauke, A.E. Campbell, R. Gamsjaeger, H. Wang, Y. Cheng, A.N. Billin, R.C. Hardison, J.P. Mackay, G.A. Blobel, Bromodomain protein Brd3 associates with acetylated GATA1 to promote its chromatin occupancy at erythroid target genes, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) E159–E168.
[25] V. Migliori, M. Mapelli, E. Guccione, On WD40 proteins: propelling our knowledge of transcriptional control? Epigenetics 7 (2012).
[26] J. Wysocka, T. Swigut, T.A. Milne, Y. Dou, X. Zhang, A.L. Burlingame, R.G. Roeder, A.H. Brivanlou, C.D. Allis, WDR5 associates with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development, Cell 121 (2005) 859–872.
[27] Y.S. Ang, S.Y. Tsai, D.F. Lee, J. Monk, J. Su, K. Ratnakumar, J. Ding, Y. Ge, H. Darr, B. Chang, J. Wang, M. Rendl, E. Bernstein, C. Schaniel, I.R. Lemischka, Wdr5 mediates self-renewal and reprogramming via the embryonic stem cell core transcriptional network, Cell 145 (2011) 183–197.
[28] H. Zhao, Y. Cao, H. Grunz, Isolation and characterization of a Xenopus gene (XMLP) encoding a MARCKS-like protein, Int. J. Dev. Biol. 45 (2001) 817–826.
[29] P.D. Nieuwkoop, J. Faber, A Normal Table of *Xenopus laevis* (Daudin), North Holland Publishing company, Amsterdam, The Netherlands, 1967.
[30] S. Rozen, H. Skaletsky, Primer3 on the WWW for general users and for biologist programmers, Methods Mol. Biol. 132 (2000) 365–386.
[31] S.R. Alappat, M. Zhang, X. Zhao, M.A. Alliegro, M.C. Alliegro, C.A. Burdsal, Mouse pigpen encodes a nuclear protein whose expression is developmentally regulated during craniofacial morphogenesis, Dev. Dyn. 228 (2003) 59–71.
[32] P.A. Gray, H. Fu, P. Luo, Q. Zhao, J. Yu, A. Ferrari, T. Tenzen, D.I. Yuk, E.F. Tsung, Z. Cai, J.A. Alberta, L.P. Cheng, Y. Liu, J.M. Stenman, M.T. Valerius, N. Billings, H.A. Kim, M.E. Greenberg, A.P. McMahon, D.H. Rowitch, C.D. Stiles, Q. Ma, Mouse brain organization revealed through direct genome-scale TF expression analysis, Science 306 (2004) 2255–2257.

[33] M. Hollmann, E. Simmerl, U. Schäfer, M.A. Schäfer, The essential *Drosophila melanogaster* gene wds (will die slowly) codes for a WD-repeat protein with seven repeats, Mol. Genet. Genomics 268 (2002) 425–433.
[34] GUDMAP, GenitoUrinary Development Molecular Anatomy Project (GUDMAP), http://gudmap.org/index.html.
[35] C.D. Stern, Neural induction: old problem, new findings, yet more questions, Development 132 (2005) 2007–2021.
[36] O. Wessely, U. Tran, *Xenopus pronephros* development—past, present, and future, Pediatr. Nephrol. 26 (2011) 1545–1551.
[37] I. Muñoz-Sanjuán, E. Bell, C.R. Altmann, A. Vonica, A.H. Brivanlou, Gene profiling during neural induction in *Xenopus laevis*: regulation of BMP signaling by post-transcriptional mechanisms and TAB3, a novel TAK1-binding protein, Development 129 (2002) 5529–5540.
[38] A.O. Perantoni, O. Timofeeva, F. Naillat, C. Richman, S. Pajni-Underwood, C. Wilson, S. Vainio, L.F. Dove, M. Lewandoski, Inactivation of FGF8 in early mesoderm reveals an essential role in kidney development, Development 132 (2005) 3859–3871.
[39] Q. Gan, P. Thiébaud, N. Thézé, L. Jin, G. Xu, P. Grant, G.K. Owens, WD repeat-containing protein 5, a ubiquitously expressed histone methyltransferase adaptor protein, regulates smooth muscle cell-selective gene activation through interaction with pituitary homeobox 2, J. Biol. Chem. 286 (2011) 21853–21864.
[40] J.H. Lee, C.M. Tate, J.S. You, D.G. Skalnik, Identification and characterization of the human Set1B histone H3-Lys4 methyltransferase complex, J. Biol. Chem. 282 (2007) 13419–13428.
[41] E.D. Zhu, M.B. Demay, F. Gori, Wdr5 is essential for osteoblast differentiation, J. Biol. Chem. 283 (2008) 7361–7367.
[42] G.R. Dressler, Epigenetics, development, and the kidney, J. Am. Soc. Nephrol. 19 (2008) 2060–2067.
[43] S.L. Rosenberg, S. Chen, N. McLaughlin, S.S. El-Dahr, Regulation of kidney development by histone deacetylases, Pediatr. Nephrol. 26 (2011) 1445–1452.